REMARKS

I. Claim Amendments

Applicants have amended the claims to better define their invention.

Claim 1 has been cancelled without prejudice. Claim 2 has been amended to an independent claim reciting "[a]n adenoviral vector comprising a DNA sequence encoding endostatin operatively linked to a promoter controlling expression of said DNA sequence, and further comprising a DNA sequence encoding a secretion signal peptide immediately 5' and fused in-frame to said DNA sequence encoding endostatin." Amended claim 2 recites the essential features of applicants' invention -- an adenoviral vector comprising a DNA sequence encoding a secretion signal peptide fused to a DNA sequence encoding endostatin and a promoter to drive the expression of the signal peptide-endostatin fusion protein. As demonstrated in the application, these elements result in a surprising and unexpectedly high level and duration of endostatin expression, as well as producing endostatin with a higher specific activity than the recombinant endostatin produced in the prior art.

Claims 28, 33, 38-41, 45 and 47-49 have each been amended to depend from now independent claim 2. Claims 43 and 44 have been amended to recite a "vector ... comprising a polynucleotide sequence encoding the amino acid sequence set forth in" SEQ ID NOs:2 and 5, respectively.

Claim 45 has been amended to recite that the claimed vector is free of at least a portion of each of the adenoviral E1 and E3 DNA sequences.

None of these amendments present new matter.

II. Claim Rejections

Applicants note with appreciation the Examiner's acceptance of the formal drawings submitted on November 22, 2002. Applicants also note with appreciation the Examiner's withdrawal of the §§102 and 103 rejections over Leboulch et al. on the basis of the 37 C.F.R. §1.131 declaration submitted on November 26, 2002.

Claims 43 and 44 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for reciting a vector (a nucleotide sequence) comprising a sequence corresponding to SEQ ID NOs that recite amino acid sequences. Applicants have obviated this rejection by adopting the Examiner's suggestion of amending the claims to recite that the vectors comprise "a polynucleotide sequence encoding the amino acid sequence set forth in" the referenced SEQ ID NO.

Claim 42 stands rejected under 35 U.S.C. §112, second paragraph, as being indefinite for reciting "the leader sequence of BM40," which, according to the Examiner, is "not set forth nor specifically defined in the specification." Applicants traverse this rejection. Paragraph 133 of the application* specifically sets forth the preparation of the BM40 basement protein leader sequence, including the specific sense and antisense complementary oligonucleotides used to create the leader (SEQ ID NOs:16 and 17) and the restriction enzymes used to trim that leader down. Figure 13 shows an amino acid sequence in which the human BM40 basement leader ("Human BM40 Signal") is clearly set forth. Thus, both the amino acid sequence of the BM40 leader and at least one polynucleotide sequence encoding that amino acid sequence are disclosed in

* References are to paragraph numbers in the corresponding published United States Application No US 2002/0115202 A1 for ease in the Examiner locating the cited portions of the specification.

the specification. Accordingly, applicants request that the Examiner withdraw this rejection.

Claim 45 stands rejected under 35 U.S.C. §112, second paragraph, as being indefinite because "the metes and bounds encompassed by the recitation 'at least the majority of' is indefinite." Applicants have obviated this rejection by amending claim 45 to delete "the majority of" and substitute therefor "a portion thereof." The amended claim language meets the requirements of 35 U.S.C. §112, second paragraph.

Claims 1, 28 and 29 stand rejected under 35 U.S.C. §102(e) as being anticipated by Crystal et al. (US2002/0076395). Applicants have obviated this rejection by canceling claim 1 and amending claim 28 to depend from amended claim 2 (claim 29 depends from claim 28). Crystal et al. does not teach or suggest the currently claimed adenoviral vector encoding a secretion signal peptide fused to endostatin, nor does Crystal et al. recognize that such a construct would result in a surprising and unexpectedly high level and long duration of endostatin expression both *in vitro* and *in vivo*. Applicants request that the Examiner withdraw this §102 rejection.

Claims 1, 28-29, 34-37, and 39-41 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Folkman et al. (WO 96/35774) and O'Reilly et al. (US Patent No. 5,854,205). The Examiner asserts that O'Reilly et al. teaches endostatin, its expression from various vectors and its anti-tumor properties against human and mouse tumors in an animal model, as well as suggesting that the endostatin encoding polynucleotide sequence may be used in gene therapy. The Examiner admits that O'Reilly does not provide specific methodologies for the delivery of endostatin. The Examiner further asserts that Folkman et al. teach that administration of anti-angiogenic

proteins is useful in treating and inhibiting angiogenesis of tumors and suggests delivery of such proteins by adenoviral vectors in gene therapy using endogenous and exogenous promoters. Thus, the Examiner concludes that it would have been *prima facie* obvious to use Folkman et al.'s suggested adenoviral delivery methods for anti-angiogenic factors to deliver endostatin taught by O'Reilly et al. Applicants traverse.

Claim 1 has been cancelled and each of claims 28-29, 34-37, and 39-41 now depends directly or indirectly from amended claim 2. Amended claim 2 requires the presence of an in-frame signal sequence immediately 5' to the endostatin coding sequence in an adenoviral vector. Neither Folkman et al., nor O'Reilly et al. teaches or suggests such a vector construct. O'Reilly et al. does not even refer to adenoviral vectors. And Folkman et al. does not refer to endostatin. Moreover, Folkman et al.'s reference to adenoviral vectors is very generalized with no teaching or suggestion of how to construct an adenoviral vector that can even express angiostatin. Accordingly, the combination of Folkman et al. and O'Reilly et al. falls far short of rendering amended claim 2 or any of the pending claims unpatentable.

Claims 1-3 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Folkman et al. (WO 96/35774) and O'Reilly et al. (US Patent No. 5,854,205) in further view of Blezinger et al. The Examiner's view of Folkman et al. and O'Reilly et al. has been set forth above. The Examiner contends that Blezinger et al. teaches the administration of a polynucleotide encoding an Ig-Kappa-endostatin fusion protein results in the production of endostatin in the serum. Thus, the Examiner concludes that it would have been *prima facie* obvious to use the fusion protein taught by Blezinger et al.

in the adenoviral delivery methods for anti-angiogenic proteins taught by Folkman et al. specifically for endostatin taught by O'Reilly et al. Applicants traverse.

Claim 1 has been cancelled. Blezinger et al. refers to the *in vitro* expression of an Ig-Kappa-endostatin fusion protein in CMV promoter-driven plasmids. Blezinger et al. also refers to the use of such plasmids amplified in *E. coli* for intramuscular injection into mice. Blezinger et al. does not teach or suggest adenoviral vectors for endostatin expression.

Further, applicants disagree with the Examiner's contention that the combination of Folkman et al. and O'Reilly et al. suggests the use of adenoviral vectors for the expression of endostatin with a reasonable expectation of success. This is because Folkman et al. and O'Reilly et al. represent work from the same laboratory. The Examiner should note that O'Reilly is a co-inventor on Folkman et al. and Folkman is a co-inventor on O'Reilly et al. Moreover, Folkman et al. has a priority date that precedes O'Reilly et al. by 6 months. Given these facts, the absence of any disclosure or suggestion in O'Reilly et al. that adenoviral vectors could be used to express endostatin belies the Examiner's assertion that one of skill in the art would be motivated to combine these two references. It is much more likely that one of skill in the art would view the absence of any disclosure of adenoviral vectors in O'Reilly et al. in light of the disclosure in Folkman et al. as a suggestion that adenoviral vectors were *not* useful for expressing endostatin for gene therapy. And even assuming that there is some motivation to combine these references (and there is none), these facts could not possibly provide one of skill in the art with a reasonable expectation of success. Thus, the combination of

Folkman et al., O'Reilly et al. and Blezinger et al., does not render claim 2 or the other pending claims obvious.

But there is even a further reason for this. Folkman et al.'s very general suggestion to use adenoviral vectors for delivery of angiostatin because they "will express gene product protein at high levels" and because "another **potential** advantage ... is the ability to achieve long-term expression of heterologous genes *in vivo*" (bolded emphasis added) is at best speculation. Folkman et al. does not show any examples that demonstrate expression of angiostatin using an adenoviral vector. And Folkman et al.'s own words demonstrate that there can be no reasonable expectation that an adenoviral vector **will** result in long-term expression of angiostatin. The Examiner's attempt to extrapolate these speculations to endostatin, even when viewed in the best light (which would require the incorrect assumption that one would combine Folkman et al. with O'Reilly et al.) would produce no more than an invitation to experiment. This is not a proper basis upon which to build an obviousness rejection.

Moreover, there is nothing in any of Folkman et al., O'Reilly et al. or Blezinger et al. that would suggest that applicants' adenoviral-based endostatin expression vectors would be capable of producing such large quantities of highly active endostatin *in vivo* over such a long period of time. Blezinger et al. found that the peak amount of recombinant endostatin in serum following intramuscular injection of mice with 240 μg of a plasmid expressing a recombinant Ig-Kappa-endostatin fusion protein was 8 ng/ml at 7 days post injection (p. 344). By 14 days post-injection, the serum level had decreased by 50% (p. 344).

In contrast, when applicants injected their adenoviral-based Ig-Kappa-endostatin fusion protein expression vector into mice, they detected serum levels of endostatin that ranged from approximately 150 to 575 ng/ml at 10 days post-injection of the vector (see Figure 8A and paragraph 120). That serum level is surprisingly and unexpectedly 19 to 72 times greater than the amount produced by the vectors of Blezinger et al. In addition, applicants observed circulating levels of endostatin of 140 ng/ml ± 64 ng/ml at 144 days post-injection in mice that survived implantation with colon adenocarcinoma (paragraph 120). Such high levels and long durations of endostatin expression could not be predicted by any combination of Blezinger et al., Folkman et al., and O'Reilly et al.

Importantly, applicants have demonstrated that the surprising and unexpected activity of their adenoviral-based vectors translates into effective anti-tumor activity. Sixty-two and a half percent of mice treated with such a vector survived for at least 53 days following colon adenocarcinoma implantation. And 25% survived for at least 188 days following implantation (paragraph 199). The efficacy of applicants' claimed endostatin-producing vectors in dramatically increasing survival following tumor implantation could not be predicted by any combination of Blezinger et al., Folkman et al., and O'Reilly et al. Accordingly, the combination of those reference does not render the presently claimed invention unpatentable. The Examiner is requested to withdraw this §103 rejection.

Claims 1 and 33 stand rejected under 35 U.S.C. 103(a) as being "unpatentable" over Folkman et al. and O'Reilly et al. in further view of Lemarchard et al. The Examiner asserts that Lamarchard et al. "teach to use recombinant adenoviral

vectors for expression transgenes under the control of the RSV promoter, therefore it would have been *prima facie* obvious to … use the RSV promoter for the expression of … endostatin with an adenoviral expression vector." Applicants traverse.

Claim 1 has been cancelled and claim 31 now depends from claim 2. These claims, as well as all of the pending claims, require that the endostatin gene be fused in frame to a signal sequence in the adenoviral vector. As set forth in detail above, neither Folkman et al., nor O'Reilly et al. teaches or suggests an adenoviral vector that expresses endostatin in any form, much less the signal sequence-endostatin fusion configuration in applicants' adenoviral vector. Lemarchand et al. provides nothing to cure the deficit of the other two references because it does not teach, suggest or even relate to an adenoviral vector encoding endostatin or a signal sequence-endostatin fusion protein. And there is nothing in Lemarchand that would lead one of skill in the art to believe that the present applicants' vectors would produce endostatin in surprisingly and unexpectedly high quantities and for long durations of time when injected into an animal. Accordingly, applicants request that the Examiner withdraw this §103 rejection.

Claim 1, 28-30, 33, 38 and 45-49 stand rejected under 35 U.S.C. 103(a) as being "unpatentable" over Folkman et al. and O'Reilly et al. in further view of Kovedsi et al. The Examiner contends that Kovedsi et al. "teach to use recombinant adenoviral vectors for expression transgenes under the control of the RSV promoter and adenoviral promoters such as the E4 promoter, and alterations to the E1-E4 regions to make replication deficient adenoviral vectors." Thus, the Examiner concludes that "one having ordinary skill in the art would have been motivated to use adenoviral vectors disclosed by Kovedsi et al. with the RSV promoter and adenoviral promoters to express heterologous

transgenes because with the deletions the vectors could accommodate larger transgenes and because the resulting vectors were replication defective" and that "there would be a reasonable expectation of success to use the recombinant adenoviral vectors disclosed by Kovedsi et al. for the expression of the endostatin sequences as disclosed by O'Reilly et al. and Folkman et al. to provide an adenoviral vector comprising an RSV promoter or adenoviral promoter operabl[y] linked to a polynucleotide sequence encoding endostatin protein and to use said vector to express said protein in a cell." Applicants traverse.

Claim 1 has been cancelled and claims 28-30, 33, 38 and 45-49 now depend directly or indirectly from amended claim 2. As stated above, claim 2 requires that the endostatin gene be fused in frame to a signal sequence in the adenoviral vector and the combination of Folkman et al. and O'Reilly et al. does not teach or suggest such a construct, nor provide any prediction of the surprising and unexpected properties of such a vector in terms of quantity or longevity of endostatin expression. Kovedsi et al. does nothing to remedy the shortcomings of the Folkman et al. and O'Reilly et al. combination. Kovedsi et al. does not relate to the expression of endostatin – either fused to a signal sequence or not. And there is nothing in the disclosure of Kovedsi et al. to suggest that the present applicants' adenoviral vectors would be so potent in expressing endostatin when injected into an animal. Thus, the Examiner's combination of Folkman et al., O'Reilly et al. and Kovedsi et al. falls far short of rendering the presently claimed invention obvious. Accordingly, the Examiner should withdraw this §103 rejection.

Claims 1, 28, 29 and 31 stand rejected under 35 U.S.C. 103(a) as being "unpatentable" over Folkman et al. and O'Reilly et al. in further view of Kovedsi et al. and in further view of Henderson et al. According to the Examiner, "Henderson et al.

teach that recombinant adenoviral vectors deficient in E3 are cytotoxic to Hep3B cells and tumors generated by Hep3B cells." Thus, the Examiner concludes that "there would have been a reasonable expectation of success to use the recombinant adenoviral vectors that express endostatin made obvious by O'Reilly et al. and Folkman et al. to infect Hep3B cells in light of the ability of Henderson et al. to infect HepB3 cells with similar recombinant adenoviral vectors which do not express a transgene." Applicant traverse.

This rejection, like the previous two §103 rejections addressed above, must fall for several reasons. First, claim 1 has been cancelled and all pending claims now require that the endostatin gene be fused in frame to a signal sequence in the adenoviral vector. Second, the combination of Folkman et al. and O'Reilly et al. – which forms the core of all of the Examiner's §103 rejections – does not teach or suggest applicants' presently claimed adenoviral vectors, nor the use thereof. Third, none of the secondary references cited by the Examiner (in particular Henderson et al. with respect to this rejection) can bridge the chasm between the Folkman et al. and O'Reilly et al. combination and applicants' claimed invention.

Henderson et al. does not relate to the expression of endostatin, nor a signal sequence-endostatin fusion protein. Thus, Henderson et al.'s teaching with respect to the use in Hep3B cells of adenoviral vectors that are unrelated to the presently claimed invention is of no import to patentability. The plain fact is that no combination of the cited references suggests to one of ordinary skill in the art the claimed invention. And therefore, no combination of the cited references could predict the surprising and unexpected activity of the claimed adenoviral vectors in expressing endostatin *in vivo*.

Applicants' adenoviral-based vectors for expressing endostatin are novel and non-obvious. They have surprising and unexpected efficacy in producing endostatin *in vivo* in terms of quantity and duration of expression. As demonstrated in the application, those properties directly translate into potent anti-tumor activity, the likes of which are not demonstrated or even suggested by any combination of prior art cited by the Examiner or known to applicants. Thus, applicants' invention, as now claimed, is patentable over the art and the pending claims should therefore be allowed.

Accordingly, applicants request that the Examiner enter the claim amendments presented herein, consider the foregoing remarks and allow the pending claims to pass to issue.

Respectfully submitted,

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